CLINICAL STUDY

Accuracy of Detecting Resistance to Carbapenems among Gram Negative Rods: Comparison of Three Methods

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Abstract

Objective

To compare the results of imipenem and meropenem susceptibility testing among multi-drug resistant (MDR) isolates of *Acinetobacter* spp. , *Pseaudomonas aeruginosa (P.aeruginosa)* and members of the *Enterobacteriacae*.

Methods

Three methods used for susceptibility testing of 210 isolates: disk diffusion (a reference method), MicroScan (MicroScan Walk Away 96 System, Dade Behring Inc. West Sacramento CA 95691, USA) and Etest (AB Biodisk Solna, Sweden).

Results

Of the 210 isolates, *Acinetobacter* spp. accounted for the majority of isolates [110(52.4%)] followed by *P .aeruginosa* ,79 (37.6%) . These isolates were more prevalent from respiratory specimens 98 (46.7%) , *Acinetobacter* spp. 60(28.6%) and *P.aeruginosa* 34(16.2%). The study has demonstrated discrepant results for carbapenems tested by MicroScan and Etest . For imipenem, the MicroScan exhibited 2.8 % very major error , major error was 10.1% but 3.9% by Etest for *Acinetobacter* spp. . Other discrepant results (minor errors) were 28.7% and 33% for MicroScan and Etest , respectively. For meropenem, minor errors were higher by MicroScan (13.6%) and Etest (21%). For *P.aeruginosa*, very major error (1.6%) was exhibited by imipenem Etest but major errors were 23% and 30.5% for both drugs by MicroScan , respectively .Minor errors were higher for both drugs by both methods (MicroScan: 15.3% to 20.8% and Etest : 34.9% to 34.2%).

Conclusion

Microbiology laboratories should consider the use of an additional confirmatory test for carbapenem susceptibility testing of clinical isolates of *Acinetobacter* spp. and *P.aeruginosa* and members of the *Enterobacteriacae*.

Key Words: Carbapenem resistance, Acinetobacter spp., *Pseudomonas aeruginosa,* MicroScan, Etest.

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Introduction

Carbapenems, such as imipenem and meropenem are a class of β-lactam antibiotics with a broadest spectrum of activity compared to other β-lactam classes in addition of being stable to the typical bacterial β-lactamase enzymes[1].Until the last few years imipenem and meropenem have been the most reliable agent for treating serious infections caused by MDR nosocomial bacteria such as *Acinetobacter baumannii* and *P. aeruginosa* [2, 3]. Although carbapenem resistance is mediated by a variety of mechanisms, it has been rarely reported [4]. However , recent reports have documented the worldwide emergence of clinical isolates of *Acinetobacter* spp, *P. aeruginosa* and other members of the *Enterobacteriacae* with acquired carbapenemases[2,5-9] .This has an important therapeutic and infection control implications as these strains are difficult to treat by β-lactamase inhibitors and resistance can spread widely into various Gram negative bacilli . Laboratory detection of resistance to carbapenems has been reported to be difficult for many reasons; low expression of such resistance , degradation of the drug , the use of automated methods for identification and susceptibility testing , in addition to the lack of standardized methods of detection [5,10,11]. Several published reports have documented problems of false resistance and false susceptibility results with imipenem and meropenem while others have shown different resistant phenotypes as well as errors of various automated systems particularly when testing β-lactam antimicrobial agents among selected Gram negative bacteria[6,10-13]. In this study, we have noticed discrepant susceptibility testing results (susceptible *vs* resistant or intermediate results) of imipenem and meropenem between disc diffusion test and the MicroScan system among MDR Gram negative bacterial isolates. Due to this inherited problem in automated systems in testing carbapenems, we compared the results of imipenem and meropenem susceptibility testing to Etest and the MicroScan to determine the degree of concordance and discrepancy between these applicable methods.

Materials and Methods

Bacterial isolates

Two hundred and ten non repetitive MDR isolates of *Enterobacteriacae* , *Acinetobacter* spp. and *P.aeruginosa* were collected during the study from August 2006 to June 2007. The isolates were from different clinical specimens (respiratory specimens, blood, body fluids, tissues urine and different swabs) submitted to the microbiology laboratory at King Khalid University Hospital (KKUH) Riyadh Saudi Arabia. KKUH is 850-bedded primary, secondary and tertiary hospital serving about two million populations. MDR are isolates that are resistant to three or all of the following classes of antimicrobial: β-lactams (ceftazidime, cefepim, piperacillin/ tazobactam), carbapenems (imipenem, meropenem) aminoglycosides (gentamicin or amikacin) and fluoroquinolones (ciprofloxacin)[14]. Identification was performed by API 20E system updated profile (bio Merieux Marcy 1'Etoile, France) and by the Micro Scan.

Inclusion criteria

We included all isolates that had resistance to carbapenems in addition to resistance to one or more classes of antibiotics during the study period.

Susceptibility testing methods

The MDR isolates were tested for susceptibility against imipenem and meropenem by the use of three methods: disk diffusion, MicroScan, and Etest. The disk diffusion test was performed using imipenem and meropenem (10 µg each), interpreted according to the Clinical and Laboratory Standards Institute (CLSI)[15] . A zone diameter of >16 mm was considered sensitive, a zone diameter of 14-15 mm as intermediate and <13mm as resistant to both antibiotics. Etest was performed on Mueller Hinton agar plate according to

manufacturer instructions and minimum inhibitory concentration (MIC) was interpreted according to CLSI criteria [15]. An MIC of 4-5 µg/l was considered sensitive and > 16 µg/l resistant to both antibiotics. An MIC reading that fell between twofold dilutions was rounded up to the next higher twofold dilution as described by the manufacturer. The colonies were inoculated into MicroScan Dried Gram negative breakpoint Combo Panel types 30 and Gram negative Combo Panel type 34 according to manufacturer's protocol. Quality control strains used included; *P.aeruginosa* (ATCC strain 27853) and *E.coli* (ATCC strain 25922). The antibiogram for other antimicrobial agents that did not exhibit any discrepant results were not included in the study. Characteristics of the type of carbapenem resistance is not performed in our laboratory

Data analysis

We calculated the very major and minor errors by comparing the testing methods to the reference methods using CLSI guidelines for verification of *in vitro* susceptibility testing [16] to evaluate the accuracy of the test and this parameter was used instead of P-value in this study. In order to have valid calculation of error, it is recommended to have at least 35 of resistant isolates for each antibiotic [17] and in our study; we tested all the resistant isolates during the study period. All the applicable statistical analysis was performed using SSPS 12.0 statistical software (SSPS Inc. Wacker Drive, Chicago).

Results

Of the 210 MDR bacterial isolates included in this study, 110 (52.4%) were *Acinetobacter* spp. and 79 (37.6%) were *P.aeruginosa* .The rest were different *Enterobacteriacae* spp. isolated in small numbers (**Table 1)**. These isolates were more prevalent from respiratory tract specimens [98 (46.7%)] from where *Acinetobacter* spp.and *P.aeruginosa* were commonly isolated. These included sputum, endotracheal aspirates and bronchial wash. Furthermore, *P.aeruginosa* was isolated from 31 (14.8 %) swabs followed by *Acinetobacter* spp. 28 (13.3%). Swabs included; wounds and other screening swabs from different body sites. These two organisms have also been commonly isolated from urine, blood, body fluids, catheter tips and tissues (**Table 1**). The results of susceptibility testing for the MDR isolates were tested using Etest and MicroScan methods and were compared to the disk diffusion (reference method) as shown in **Table 2**. Twenty five isolates of the *Acinetobacter* spp. were susceptible to imipenem compared to 16 by Etest and 10 by MicroScan . Seventy one of *Acinetobacter* spp. isolates were resistant to imipenem by disk diffusion compared to 53 and 57 by the Etest and the MicroScan, respectively (7 isolates were not tested by Etest and 2 by the MicroScan). Seventy one isolates of *P.aeruginosa* were resistant to imipenem by MicroScan. Resistance to meropenem among *Acinetobacter* spp. was similar between disk diffusion and Etest (89 isolates each) compared to MicroScan (64 isolates) while 9 isolates have shown intermediate susceptibility to merpenem, 26 isolates were so by Etest and 14 isolates by MicroScan. Regarding *P.aeruginosa* , only one isolate was susceptible to imipenem by MicroScan but 22 isolates were susceptible by disk diffusion and 16 isolates by Etest .For meropenem, 25 *P.aeruginosa* isolates were resistant to meropenem by disk diffusion while 9 isolates were resistant by E test. No resistance detected by disk diffusion or Etest were detected among other isolates. Although a tendency toward carbapenem resistance results was noticed when *Acinetobacter* spp. and *P.aeruginosa* were tested by MicroScan, resistance to imipenem was much higher for *P.aeruginosa* by MicroScan compared to *Acinetobacter* spp. Conversely, imipenem intermediate susceptibility was much lower for *P.aeruginosa* by MicroScan (6 isolates) compared to *Acinetobacter* spp.(41 isolates). Forty seven *P.aeruginosa* isolates were susceptible to meropenem by disk diffusion while only 22 isolates were susceptible to imipenem. For the other isolates, the majority were susceptible to both drugs by

disk diffusion and Etest compared to MicroScan except for *K.pneumoniae* where two of the three isolates were resistant to imipenem by disk diffusion and Etest while all were susceptible to meropenem by the two methods (not tested by MicroScan) and Provedencia spp. were susceptible to both drugs by all methods but one isolate was resistant to meropenem by MicroScan. Considering disk diffusion method as a reference, the accuracies of MicroScan and Etest for testing carbapenems against MDR isolates is shown in **Table 3**. The result of testing *Acinetobacter* spp. against imipenem by MicroScan exhibited 2.8 % very major error (false susceptible) compared to 0 % by meropenem . For other isolates there was no significant difference due to small number of isolates tested. Major error (false resistant) for *Acinetobacter* spp. was 10.1% by MicroScan while 3.9 % by Etest. There was 0% major error for meropenem by Etest for all isolates. Minor error (any other discrepant results with an MIC between 6-16 µg/l) was nearly similar for MicroScan and Etest, 28.7% and 33% respectively. For meropenem, no very major error was detected by MicroScan while only 1% by Etest. In contrast, when *Acinetobacter* spp was tested by Etest it produced no major error while 1.94 % rate of major error by MicroScan. However, minor error was higher by Etest (21%) compared to MicroScan (13.6%). For *P.aeruginosa,* there was no very major errors when the bacteria was tested by MicroScan for both drugs but only for meropenem by Etest while there was 1.6% very major error when the imipenem was tested by Etest. In contrast, major error was exhibited when both carbapenems were tested by MicroScan, 23% and 30.5%. Minor error was higher for imipenem and meropenem (MIC was intermediate) when *P.aeruginosa* was tested by Etest (34.9%-34.2%) compared to MicroScan (15.3% -20.8%). Regarding other isolates, major errors were noticed for *Enterobacter* spp. ,*Escherichia coli* (*E.coli*) and *Klabsiella pnaumoniae* (*K.pneumoniae*) , 55.5%,50% and 33.3% respectively, when imipenem was tested by MicroScan and 57.1% major error for *Enterobacter* spp. when meropenem was tested by Etest but 28.6 % minor error when meropenem was tested by MicroScan.

Table 1: Clinical isolates from different clinical sites

Bacteria	Respiratory No (%)	Swabs No (%)	Urine No (%)	Catheter tips No (%)	Blood No (%)	Body fluids No (%)	Tissues No (%)	Total No (%)
Acinetobacter spp.	60 (28.6)	28 (13.3)	12 (5.7)	5 (2.4)	2 (1.0)	2 (1.0)	1 (0.5)	110 (52.4)
P.aeruginosa	34 (16.2)	31 (14.8)	5 (2.4)	5 (2.4)	-	2 (1.0)	2 (66.6)	79 (37.6)
Enterobacter spp.	2 (1.0)	6 (2.9)	1 (0.5)	-	-	-	-	9 (4.3)
E.coli	1(0.5)		3 (1.4)	-	-	-	-	4 (1.9)
K.pneumoniae		-	3 (1.4)	-	-	-	-	3(1.4)
Provedencia spp.	-	-	1 (0.5)	-	1(0.5)	-	-	2 (1.0)
Citrobacter spp.	1 (0.5)	-	1 (0.5)	-	-	-	-	2 (1.0)
S. marcescens	-	-	1 (0.5)	-	-	-	-	1(0.5)
Total = 210	98(46.7)	65 (31)	27 (12.9)	10 (4.8)	3 (1.4)	4(1.9)	3 (1.4)	210 (100)

Table 2: Results of comparison of Etest and MicroScan methods to disk diffusion (reference method)

		Imipenem (N)				Meropenem (N)			
Bacteria	**Methods**	**S**	**I**	**R**	**NT***	**S**	**I**	**R**	NT*
Acinetobacter spp.	DD** Etest MicroScan								
P. aeruginosa	DD** Etest MicroScan								
Enterobacter spp.	DD** Etest MicroScan								
E. coli	DD** Etest MicroScan								
K. pneumonia	DD** Etest MicroScan								
Provedencia spp.	DD** Etest MicroScan								
Citrobacter spp.	DD** Etest MicroScan								
S. marcescens	**DD** **Etest** **MicroScan**								

NT*; not tested, DD**; disk diffusion, S; sensitive, I; intermediate, R; resistant.

Table 3: Accuracy of detecting resistance to carbapenems among *Acinetobacter* spp., *P.aeruginosa* and common *Enterobacteriaecae* isolates using disk diffusion as reference method

Methods	Carbapenems and error type	*Acinetobacter* spp. (% error)	*P.aeruginosa* (% error)	*Enterobacter* spp.(%error)	*E.coli* (%error)	*K.pneumonie* (% error)
MicroScan	**Imipenem**					
	very major	2.8		0	0	0
	major	10.1	23	55.5	50	33.3
	minor	28.7	15.3	11.1	25	0
	Meropenem					
	very major		0	NT*	NT*	NT*
	major	1.94	30.5	57.1	0	0
	minor	13.6	20.8	28.6	0	0
Etest	**Imipenem**					
	very major	0	1.6		0	0
	major	3.9	0		25	0
	minor	33	34.9	11.1	0	0
	Meropenem					
	very major	1		NT*	NT*	NT*
	major	0		0	0	0
	minor	21	34.2	0	0	0

NT*; not tested

Discussion

In many diagnostic microbiology laboratories use automated systems such as the MicroScan for quick identification and susceptibility testing of commonly isolated bacteria. These systems have many advantages including testing large number of clinical specimens and decreasing the in-laboratory turnaround time. However, the occurrence of discrepant results of susceptibility testing of important nosocomial pathogens to carbapenems tested by the MicroScan had a serious concern for treatment of very ill patients as well as the control of dissemination of resistance in hospital settings. The results of this study have demonstrated that the MicroScan has produced discrepant results of susceptibility testing among clinically significant isolates of *Acinetobacter* spp. and *P.aeruginosa* and some enteric MDR isolates. Imipenem and meropenem Etest results have also produced conflicting results for *Acinetobacter* spp. , for imipenem the results were closely similar to MicroScan however, for meropenem , the results were closely similar to disk diffusion .The sensitivity and specificity of disk diffusion method could not be calculated for two reasons. First, it was the reference method. Second, sensitivity and specificity of disk diffusion method could not be calculated due to unavailability in our laboratory of other gold standard methods like agar dilution or microdilution to compare with. A pseudo-outbreak of imipenem resistant *Acinetobacter baumannii* has been reported from Greece using Vitek automated system while by disk diffusion all isolates were defined in the susceptible range [8]. For *P.aeruginosa*, the results of Etest in our study yielded the highest discrepant results with imipenem and meropenem (34.9% and 34.2 % minor errors , respectively). In contrast to our results, Steward *et al* have shown that the MicroScan has resulted in 14.8% major error and 29.6% minor error when tested *P.aeruginosa* against imipenem [11]. For isolates of *Enterobacteriacae* , minor errors for both carbapenems were observed by agar dilution, disk diffusion ,Etest, MicroScan and Vitek compared to broth microdilution ranged from 1.1% to 8.4% and minor error rates were higher for imipenem than for meropenem [11] . The higher percentage of errors observed for *P.aeruginosa* was resulted from more isolates of *P.aeruginosa*

that had an MIC test results that clustered around carbapenem breakpoints than members of the *Enterobacteriacae* [11]. Sader *et al* observed a slight tendency toward more resistant results with *P.aeruginosa* when imipenem was tested with the Vitek , 72% compared to 68% by MicroScan and consensus ,71% [18.] Moreover, minor error was 10 % when *P.aeruginosa* was tested with MicroScan compared to 8% and 11% by Vitek 2 and Vitek systems, respectively [18]. In a multicenter laboratory evaluation of bioMerieux Vitek system for antimicrobial susceptibility testing versus members of the family *Enterobacteriacaea* and *P.aeruginosa* have demonstrated the importance of inoculum size as a determinant of the accuracy of susceptibility testing results with Vitek system i.e. when the inoculum density is fourfold higher than the recommended by the manufacturer ,high rates of false resistance results were obtained with cell wall active agents versus *Enterobacteriacae* and *P.aeruginosa* [10]. Daly *et al* reported that resistance to imipenem among *P.aeruginosa* isolates may be due to the zinc concentration in Mueller Hinton agar that has been shown to affect that MIC of imipenem and susceptibility testing by other methods [19]. In addition *P.aeruginosa* requires higher concentrations of imipenem than those required by members of the *Enterobacteriacae* [19]. Regarding other isolates, when *Enterobacter* was tested in our study ,the number of isolates that have been susceptible to imipenem and meropenem by disk diffusion have dropped from 9 for both drugs and 8 and 9 by Etest to 3 and 1 by MicroScan, respectively .A false susceptible results for *K.pneumoniae* isolates when tested carbapenems by MicroScan system was reported by Bratu *et al* which were attributed to inoculum size as well [5].Although Tenover *et al* have reported variability in detecting imipenem resistance among *K.pneumoniae* with automated systems , he also reported that the MicroScan and BD Phoenix systems produced results that were more consistent with reference testing systems than those with the Vitek and Sensititre AutoReader systems [4] . In agreement with our study, he also reported difficulties in determining the results with Etest because of the presence of colonies within the zones of inhibition and disk diffusion was used to confirm carbapenem resistance results particularly for meropenem in *K.pneumoniae* isolates [4] .Earlier study by O'Rourke *et al* have demonstrated that false resistance to imipenem was due in part to the lost of its potency by using Sensititre microdilution MIC that used custom lots of trays stored at room temperature [20]. Imipenem was reported to be stable at -70^0 C for up to 1 year but can deteriorate over time even in a pre-dried format stored as recommended by the manufacturer [21]. This could be the case in our study, although we used an inoculum size and storage conditions as recommended by the manufacturer. Other unknown factors may also be attributed under our laboratory conditions. In the study by Steward *et al* ten laboratories under-reported imipenem resistance in *Serratia marcescens* (S. *marcescens*) isolates which was due in part to the isolate itself where carbapenem resistance in *S. marcescens* is due to different mechanism [11]. Other isolates in our study including *S.marcescens* were very few in numbers that it may not give an accurate estimation of discrepant results. It seems as well that the presence of different mechanisms mediating resistance to carbapenems among isolates of *Acinetobacter* spp. , *P.aeruginosa* and selected members of the family *Enterobacteriacae* are further attributed to inaccurate detection of resistance by microbiology laboratories . These mechanisms include impermeability ,the production of metallo-β-lactamases, porin changes and changes in penicillin – binding proteins that require routine screening using different methods such as EDTA disk screen or PCR for confirmation [4,6,11] . Some microbiology laboratories in the developing world might not be faced with such a problem particularly those that do not use the automated systems.

In conclusion, inaccurate detection of carbapenems resistance among *Acinetobacter* spp. and *P.aeruginosa* and members of the family *Enterobacteriacae* by automated

systems and Etest is worldwide problem. We recommend that diagnostic microbiology laboratories should be aware of the problem and reevaluate their susceptibility testing methods and consider using an additional nonautomated method such as disk diffusion to confirm carbapenem nonsusceptibility. Changes in susceptibility profiles of many hospital pathogens demand the continuous improvement of automated systems for accurate reading of susceptibility testing results.

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